



REVIEW

Angiogenesis and the tumour hypoxia response in prostate cancer: A review

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Abstract The formation of new blood vessels, angiogenesis, is a highly-regulated active process that is critical for the development of the normal and malignant prostate. The vascular endothelial growth factor (VEGF) system assumes a critical role in the angiogenic process. Angiogenesis is a prerequisite for the expansion of solid tumours beyond 1–3 mm³ and is stimulated in response to a hypoxic environment. This review discusses the process of angiogenesis and the key angiogenic mediator, VEGF, and their role in tumour progression and metastasis. A better understanding of the mechanisms behind angiogenesis will ultimately lead to the development of new anti-angiogenic agents in the management of prostate cancer.

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Introduction

Prostate cancer is the most commonly diagnosed male malignancy in Europe and the USA. In Europe more than 10,000 men are found to have prostate cancer and 35,000 men die from the disease per year.^{1,2} Prostate cancer is likely to achieve an even greater incidence as populations continue to age and the mortality from other diseases decreases.

New vasculature is recruited from pre-existing vessels by a process called angiogenesis,³ and it is particularly relevant to the pathology of virtually all human tumours.⁴ Developing tumours require new vasculature as they grow in order to ensure a constant supply of nutrients and oxygen and to allow for the elimination of metabolic waste.⁴ Choy and Rafii⁵ suggested that angiogenesis is also a prerequisite for tumour progression. The angiogenic process in solid tumours is crucial for advanced tumour growth⁶ and progression to a metastatic state.⁷ It has been proposed that microvessel density (MVD) is an indicator of biological aggressiveness and metastatic potential in many primary tumours.⁸

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Immobilizing the angiogenic process could augment the effects of chemotherapy and radiation by limiting the tumour to a dormant state of low metastatic potential⁹; hence, interest in anti-angiogenic therapies has increased dramatically.¹⁰ Angiogenesis is initiated in quiescent endothelial cells following a shift in the balance between endogenous angiogenesis-inhibitory factors and angiogenesis-promoting factors⁴ (Table 1). A shift in the equilibrium to a pro-angiogenic state occurs at an early to mid-stage in tumour development. This leads to activation of an 'angiogenic switch' and, consequently, the formation of new vasculature.¹¹ Growth factors up-regulated during angiogenesis, in particular basic fibroblast growth factor (bFGF)¹² and VEGF,¹³ have been identified and may also be important as therapeutic targets and/or molecular indicators of disease stage. Raised levels of VEGF are produced in the malignant prostate compared to benign prostatic hyperplasia.¹⁴ VEGF is a key mediator of both normal and abnormal angiogenesis due to its capacity to stimulate virtually every step in the angiogenic process.^{15,16} VEGF expression in human prostate stroma can be stimulated by androgens, thus supporting the hypothesis that androgen ablation affects prostate tumours through the inhibition of angiogenesis.⁵

Angiogenesis

Blood vessels are endothelial-lined tubes that provide tissues with oxygen and nutrients and remove their metabolic waste products while also

producing a spectrum of growth factors (Table 1) with paracrine effects on surrounding cells. Thus these vessels may support both normal and malignant tissue growth by providing a sufficient blood supply and by secreting growth factors.

The mechanism of angiogenesis (Fig. 1)

The growth of new capillaries from existing blood vessels is a complex multi-step process involving extracellular matrix components. It begins with an enlargement of the parent vessel, which then sprouts or divides by intussusception or bridging, and subsequently splits into individual capillaries.¹⁷ By far the most common, sprouting angiogenesis, involves activation, migration and proliferation of endothelial cells, which ultimately assemble into solid cords that acquire a lumen and attach to existing vessels.¹⁷ The process of angiogenesis includes a maturation phase where periendothelial cells, such as smooth muscle cells and pericytes, encapsulate and stabilise the vessel by inhibiting endothelial cell proliferation and migration and enhance the formation and production of extracellular matrix.¹⁸ Whether angiogenesis occurs by sprouting, intussusception, or bridging, the above process protects new vessels from rupture and regression as well as endowing vessels with the viscoelastic and vasomotor properties necessary to accommodate changing needs in tissue perfusion.¹⁷

The angiogenic process is usually tightly controlled (i.e. turned on for a short period and then

Table 1 Angiogenic factors in prostate cancer

Route	Pro-angiogenic	Anti-angiogenic
Exogenous agents		Linomide Carboxyamido-triazole Neutralizing antibodies Fumagillin analogue MMP inhibitors COX-2 inhibitors
Endogenous agents	Matrix metalloproteinases (MMPs) VEGF Basic fibroblast growth factor 2 (bFGF-2) Fibroblast growth factor 4 (FGF-4) Transforming growth factor β 1 (TGF- β 1) Interleukin 8 (IL-8) Interleukin 6 (IL-6) Interleukin 1 β (IL-1 β) Cyclooxygenase 2 (COX-2) Nitric Oxide (NO) Tumour necrosis factor (TNF), Insulin growth factor 1 (IGF-1)	Tissue inhibitor of metalloproteinase 1 (TIMP-1) Interleukin-10 (IL-10) Angiostatin Endostatin Prostate-specific antigen (PSA) Interferon (IFN)

completely inhibited)¹⁹ by physiological means; uncontrolled angiogenesis can contribute to several pathological processes such as, rheumatoid arthritis, diabetic retinopathy and neoplastic growth.

VEGF expression correlates with malignant potential in the prostate

The malignant potential of prostate tumours is correlated to VEGF expression with aggressive tumours expressing higher levels of VEGF.²⁰ In mice models, when the activity of VEGF is blocked by antibodies or receptor signalling inhibitors, decreased vascularisation, suppression of tumour growth, and metastasis results.²¹ VEGF expression in tumours, just as in normal prostate tissue, also appears to be regulated by androgens.²² A positive correlation has been found between VEGF expression and vascular density.²³ Immunohistochemical techniques (IHC) used by Ferrer et al.²⁴ demonstrated that human prostate cancer cells stained positively for VEGF and correlated with microvessel density (MVD). They also found that benign prostatic hyperplasia (BPH) and normal prostate cells display little VEGF staining and vascularity.¹⁴

Haggstrom et al.²⁰ demonstrated that VEGF expression is associated with metastatic capability, by enhancing the hyperpermeability of vessels, endothelial cell proliferation, and endothelial cell migration. In Dunning tumours in rats, VEGF mRNA and serum protein are highest in metastatic sublines in comparison with the non-metastatic subline.²⁰ Duque et al.²⁵ also demonstrated that plasma and urine VEGF levels are higher in patients with metastatic prostate cancer than in patients

with localized disease or healthy controls. Salven et al.²⁶ also showed that circulating VEGF is higher in patients with disseminated cancer than in the normal population. A randomized controlled trial in hormone refractory patients found that low pre-treatment urine VEGF levels were predictive of improved survival²⁷; furthermore, Jose et al.²⁸ demonstrated that patients with plasma VEGF levels greater than 18 pg/ml were 10 times more likely to have metastases and that patients with PSA levels greater than 20 ng/mL had significantly higher VEGF levels than patients with PSA levels less than 20 ng/mL. They also found a trend for VEGF to be higher in patients with high Gleason sums (8–10).²⁸

Hypoxia stimulates tumour angiogenesis

The rapid growth of cancerous cells within a tumour is seldom complemented by the development of vascularisation sufficient to support the growing tumour mass; hence most human tumours encounter hypoxia early in their development.²⁹ This event is potentially advantageous to the patient as tumour cells starved of oxygen are much less likely to multiply and are increasingly liable to undergo apoptosis. The tumour's inability to attract and develop a blood supply makes more likely to remain dormant.³⁰ However, the activation of a tumour hypoxic-response system alters its metabolism, improving survivability under low-oxygen conditions and increasing its angiogenic potential by upregulating the production of critical angiogenic factors, particularly VEGF³¹ (Fig. 2), thereby improving the tumour's potential for attracting

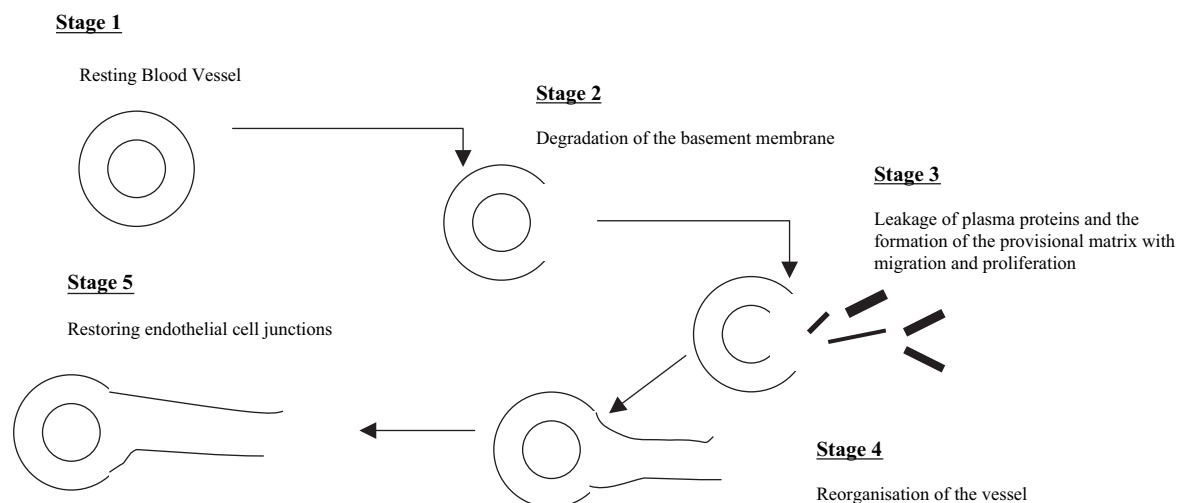


Figure 1 A diagram to show the different stages of angiogenesis.

increased oxygenation. This up-regulation of angiogenesis 'switches' the tumour from a prevascular (dormant) to a vascular (exponential growth) phase.¹⁹

HIF1 α protein has been found to be over-expressed in 13 of 19 different solid tumour types, including prostate cancer.³² Zhong et al.³² demonstrated HIF1 α positivity in nine out of 11 prostate cancer specimens examined, while none was seen in benign tissue specimens. The authors also correlated HIF1 α expression with both uncharacteristic p53 growth and increased cell proliferation activity in the tumours. Salnikow et al.³³ tested the functional activity of HIF-1 in human prostate cell lines from normal epithelial cells (PrEC), hormone dependent LNCaP, hormone-independent DU-145 and PC-3, and highly metastatic PC-3M cancer cells. The authors demonstrated that HIF-1-stimulated transcription and protein expression was lowest in the PrEC (normal epithelial cell) and LNCaP (hormone dependent) cells, and highest in the PC-3M cells (highly metastatic). Given also that high HIF-1 expression correlates with loss of p53 function and desensitization to p21 inhibition, it is reasonable to conclude that a hypoxic phenotype is associated with aggressive cancer behaviour.³³

Huss et al.³⁴ performed IHC and in situ analyses of tissue specimens using a transgenic mouse model to investigate the relationship between HIF-1, VEGF, and angiogenesis. They suggested that there were two distinct angiogenic events involved in the development and progression of prostate cancer. The first angiogenic "initiation switch" is associated with increased expression of HIF-1 and VEGF receptor plus recruitment and amplification of intraductal vasculature.³⁴ The second angiogenic "progression switch" is associated with an increased level of expression of VEGF protein in prostatic tissues as well as in the sera of mice harbouring advanced, poorly differentiated, and androgen-independent tumours.³⁴ This implies that the abnormally raised expression of HIF-1 protein in prostate tumour cells may have the ability to make these cells more aggressive via mechanisms of both androgen resistance and angiogenesis promotion. Increased levels of hypoxia, as measured in patients, are directly correlated with raised levels of expression of VEGF.³⁵ There are many interacting mechanisms for this relationship. For example, HIF-1 binding to *fms*-like tyrosine kinase-1 and fetal liver kinase 1 is known to induce VEGF expression.³⁶ Studies have also shown that in cancer cell lines, HIF-1 expression is

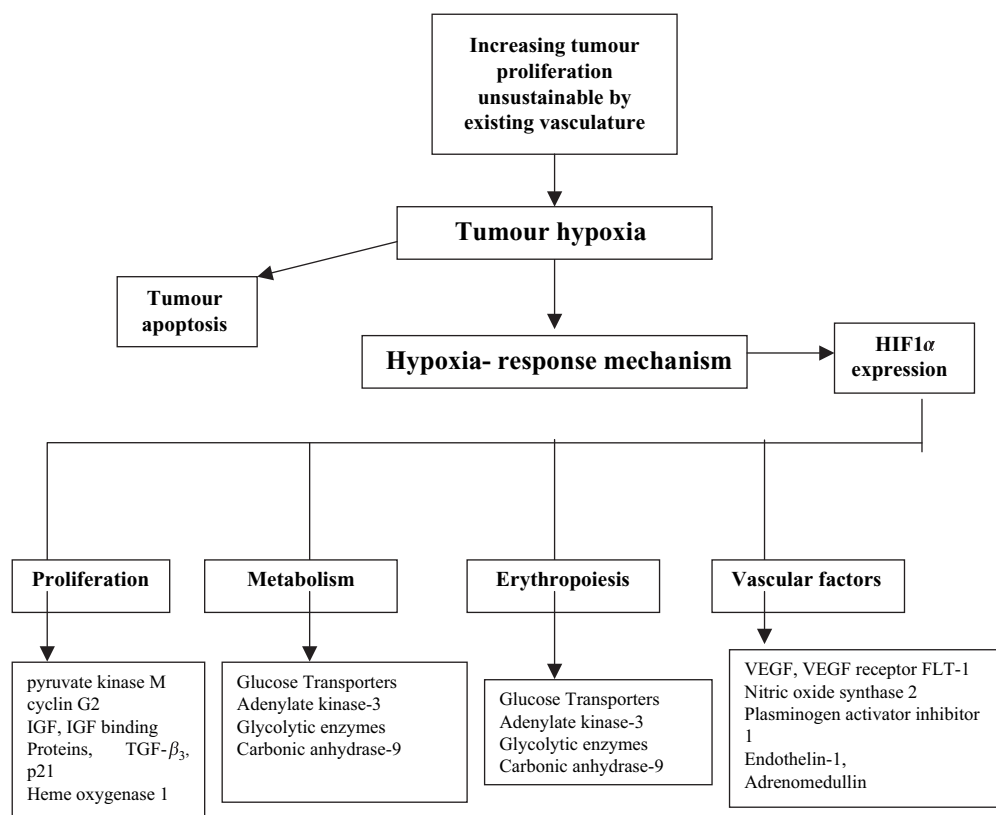


Figure 2 The hypoxia-response mechanism in the development of prostate cancer.

positively regulated by the PI3/AKT/FRAP signal transduction pathway, thus resulting in enhanced VEGF production.³⁷ This implies that, in addition to hypoxia, mutations in the tumour suppressor gene PTEN could contribute to the increased VEGF expression observed in patients. Cvetkovic et al.³⁸ proposed that prostate tumour cells adapt to decreased pO₂ levels by transcription of HIF-1, which causes VEGF production, ultimately leading to an enhanced angiogenesis with its consequent effects on the growth and the spread of such tumours.

Nitric oxide, cytokines, and growth factors can also regulate VEGF

As well as hypoxia, VEGF is regulated by many growth factors and cytokines at pre-transcriptional,³⁹ transcriptional,⁴⁰ and post-transcriptional levels.⁴¹ Fidler⁴² suggested that those molecules that do not stimulate angiogenesis directly can modulate angiogenesis by altering VEGF expression in specific cell types and exerting indirect angiogenic or anti-angiogenic effects. Molecules that can initiate VEGF production include fibroblast growth factor 2 (FGF-2),⁴³ FGF-4,⁴⁴ platelet derived growth factor (PDGF),⁴⁵ tumour necrosis factor (TNF),⁴⁶ transforming growth factor β (TGF- β),⁴⁷ insulin growth factor-1 (IGF-1)⁴⁸ and IL-6.⁴⁹

Nitric oxide (NO) and VEGF up-regulate each other's production via a positive feedback loop mechanism.⁵⁰ NO contributes to the blood vessel permeability effect of VEGF and VEGF-stimulated vasodilatation. Uotila et al.⁵¹ found that NO synthetase-2 has an elevated expression in both prostate cancer and prostate intraepithelial neoplasia (PIN) compared to normal prostate tissue. The VEGFR-2 can be up-regulated by VEGF in an autocrine and/or paracrine mechanism and it has also been suggested that VEGF autocrine stimulation may coincide with progression to a malignant phenotype.^{52,53}

Recently, *ras*, *raf*, and *src* gene products have been implicated as signalling intermediates in the induction of VEGF transcription.⁵⁴ The enhanced expression of the activated forms of these genes is accompanied by marked elevation of both VEGF mRNA.⁵⁵ VEGF expression is critical for *ras*-mediated tumorigenesis, while loss of expression causes dramatic decrease in vascular density and vascular permeability as well as an increase in tumour cell apoptosis.⁵⁶ *ras*, *raf* and *src* activating mutations are unusual in human prostate cancer, but may be major signalling molecules by which circuits can be corrupted.⁵⁷ Any factor that stimulates *ras*-, *raf*-,

and *src*-mediated signalling pathways may facilitate tumour angiogenesis via VEGF induction.

Conclusion

Angiogenesis is the key process in the development of tumour vasculature for virtually all solid malignancies including prostate cancer. It is a complex process with many stimulatory and inhibitory factors, most importantly VEGF, linked together in poorly defined pathways. Anti-angiogenic therapies (e.g. angiostatin inducers, VEGF inhibitors, and cyclo-oxygenase inhibitors) have been found to be successful in animal and cell line models, and are currently under investigation in phase 2 clinical trials. These molecules are being evaluated for both efficacy and their toxicity profiles. As angiogenesis is involved at a very early stage in prostate tumour development, such strategies may prove useful in the management of early disease. Prostate cancer has a well defined pre-neoplastic stage and it may be that anti-angiogenic strategies will be employed in individuals with high-grade PIN in an attempt to prevent the development of invasive carcinoma. As the management of high-grade PIN is currently highly controversial with no definite successful strategy, such chemopreventive therapies are badly needed.

Tumour cell hypoxia, via HIF-1, appears to be a crucial stimulant for the angiogenic process to allow tumour growth beyond a few millimetres as well as the development of metastasis. Although the tumour hypoxia response mechanism leads to a multitude of downstream effects, it is angiogenesis that is most crucial and also most susceptible to molecular manipulation. Hence specific attempts to develop targets for hypoxia have been superseded by the development of anti-angiogenic molecules. Improved understanding of the molecular mechanisms involved in angiogenesis and tumour hypoxia-response will allow for future targeted therapies to be developed that may, either alone or in combination with established modalities like chemotherapy, surgery, and radiotherapy, provide improved cure and control rates over current therapeutic strategies. Such combination strategies are currently the subject of various clinical phase 2 trials in colorectal and breast cancer. Once these trials plus those investigating the biological effect of anti-angiogenic therapies in prostate cancer report, further trials investigating chemoprevention in high-grade PIN patients as well as the use of combination treatments in those with established disease can be advocated.

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